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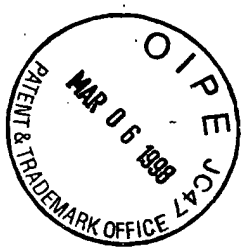

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Targeting of IgMκ Antibodies to Oligodendrocytes Promotes Central Nervous System Remyelination

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Abbreviations: TMEV, Theiler's murine encephalomyelitis virus; mAb, monoclonal antibody; CNS, central nervous system; Ig, immunoglobulin; MBP, myelin basic protein.

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ABSTRACT

We previously identified the remyelinating activity of a natural IgM/κ oligodendrocyte-reactive autoantibody (SCH94.03) using a virus-induced murine model of multiple sclerosis. This antibody could function directly by binding a surface receptor or indirectly by targeting the immune system to the lesion. We now describe a second mouse IgM/κ monoclonal antibody (mAb)(SCH79.08) raised against normal mouse spinal cord homogenate, which reacts with myelin basic protein and also promotes remyelination. As these two mAbs recognize different oligodendrocyte antigens, several previously identified oligodendrocyte-reactive IgM/κ mAbs (O1, O4, A2B5, and HNK-1), each with distinct antigen specificities, were evaluated and found to promote remyelination. In contrast all other IgM/κ mAbs that did not bind to oligodendrocyte-associated antigens had no remyelination promoting activity. One of these, the IgM/κ CH12 mAb which shares variable region cDNA sequences with SCH94.03 except for amino acid differences in the complementarity-determining region 3 in both heavy and light chains did not bind oligodendrocytes and did not promote remyelination. The fact that multiple oligodendrocyte-reactive antibodies with distinct antigen reactivities induce remyelination argues against direct activation by a unique cell surface receptor. These findings are consistent with the hypothesis that binding of mAbs to oligodendrocytes in the lesions induces myelin repair through indirect immune effector mechanisms initiated by the μ-chain. Importantly, these studies suggest that oligodendrocyte-reactive natural autoantibodies may provide a powerful and novel therapeutic means to induce remyelination in multiple sclerosis patients.

Demyelination in association with inflammation is the primary structural abnormality in multiple sclerosis (MS). Spontaneous remyelination is limited in the central nervous system (CNS), in part because oligodendrocytes are considered to be postmitotic cells. However, spontaneous remyelination is observed at the edge of MS plaques (1). Studies have shown that oligodendrocyte/type-2 astrocyte (O-2A) progenitor cells persist in the adult CNS and proliferate (2, 3). Alternatively it has been shown that mature oligodendrocytes can be induced to dedifferentiate and proliferate under the influence of neurons (4) or basic fibroblast growth factor (5, 6) providing another cellular mechanism for remyelination.

One of the major goals for the treatment of MS is to develop strategies to promote remyelination. Two alternative strategies have been considered, a) surgical transplantation of progenitor or mature oligodendrocytes into the plaques or b) pharmacological approaches to augment the potential for endogenous myelination. Even though oligodendrocyte transplantation has been shown to be partly effective in experimental demyelination and dysmyelination animal models (7), this strategy will likely not be feasible in human MS patients with multifocal disease. To date the only strategy shown *in vivo* to enhance endogenous myelination has been the use of natural germline antibodies (Abs) which react to CNS antigens. This approach is particularly attractive because it can be translated from the bench to the bedside rapidly as therapy for human MS (8, 9). We demonstrated that a mouse monoclonal antibody (mAb) raised against normal mouse spinal cord homogenate (SCH), designated SCH94.03, enhanced CNS remyelination in the Theiler's murine encephalomyelitis virus (TMEV) model of MS (10). SCH94.03 belongs to the IgMκ subclass, is highly polyreactive against known and unknown protein antigens including cytoskeletal proteins, and is encoded by unmutated immunoglobulin (Ig) germline genes confirming that SCH94.03 is a natural autoantibody (11, 12). Of unique importance SCH94.03 recognizes an unidentified surface antigen on oligodendrocytes (13).

Two major hypotheses have been proposed by which SCH94.03 promotes remyelination. 1) The mAb may bind to a unique receptor on the surface of oligodendrocytes to induce myelination. The hypothesis would predict that only a limited repertoire of Abs with unique

specificity would function. 2) The mAb may work by binding to damaged oligodendrocytes and/or myelin which trigger a cascade of events by other resident CNS cells (i.e., astrocytes, microglia, or neurons) which in turn enhance myelin repair. An attractive hypothesis is that binding to damaged oligodendrocytes and myelin may enhance opsonization and clearing of CNS debris by macrophages, thus allowing for the normal process of remyelination to ensue. This hypothesis would predict that many polyreactive autoantibodies with specificity to oligodendrocytes and/or myelin would be effective. To address these hypotheses and the mechanism for Ab-mediated CNS remyelination, we set out to generate additional mAb which promotes CNS remyelination in TMEV model and compared its specificity with SCH94.03.

MATERIALS AND METHODS

Virus and Animals.

The Daniel's strain of TMEV was used for these experiments. Female SJL/J mice from the Jackson Laboratories were used after one week rest. Mice from 4-6-week of age were injected intracerebrally with 2×10^5 plaque forming units (PFU) of TMEV in a 10 µl volume. Handling of all animals was in accordance with the institutional guidelines prescribed by the National Institutes of Health and Mayo Clinic.

MAb production and screening.

Hybridomas were generated and screened as described (10). SJL/J mice were immunized with normal mouse SCH in incomplete Freund's adjuvant and their splenocytes were fused with NS-1 myeloma cell. Hybridoma supernatants which showed high binding to SCH by ELISA were further screened for their ability to promote remyelination in the TMEV model. Therefore the Abs were screened based on the their ability to promote CNS remyelination rather than for a unique antigen specificity. IgMκ Abs were purified from hybridoma culture supernatants by ammonium sulfate precipitation and dialysis against phosphate buffered saline (PBS) plus low ionic strength precipitation, or by affinity chromatography using goat anti-mouse IgM Ab (µ-chain specific; Jackson Immunoresearch) bound to carbonyldiimidazole-activated cross-linked agarose (Reacti-Gel 6X matrix, Pierce).

Hybridomas and mAb preparation.

Hybridoma A2B5, HNK-1, and R24 were purchased from American Type Culture Collection (ATCC). Hybridoma O1 and O4 were the gift from S. E. Pfeiffer (University of Connecticut, Farmington, CT). These hybridomas were cultured in RPMI 1640 supplemented 10% fetal calf serum (Hyclone) and 2×10^{-2} mM ß-mercaptoethanol. B cell lymphoma CH12 (CH12.Lx) was provided by Dr. G. Haughton, University of North Carolina, Chapel Hill, NC). To obtain secreted IgM from CH12 lymphoma, CH12.Lx cells were stimulated with 50 µg/ml of

lipopolysaccharide (Sigma). MAb O1, O4 and HNK-1 were purified from hybridoma culture supernatant by ammonium sulfate precipitation and dialysis against PBS plus low ionic strength precipitation. MAb A2B5 and CH12 were purified by affinity chromatography. R24 was purified by protein A column. The purity of these mAbs was examined by SDS-polyachrylamide gel electrophoresis and the immunoreactivity of these mAbs was examined by immunostaining with rat oligodendrocytes. Control hybridoma XMMEN-OE5 producing anti-bacterial lipopolysaccharide IgMκ Ab was purchased from ATCC. Clarified ascites of control ABPC22 IgMκ mAb was purchased from Sigma. Both IgMκ mAbs were purified by affinity chromatography using goat anti-mouse IgM Ab. The purity of the mAbs was confirmed by SDS-polyacrylamide gel electrophoresis.

MAb treatment and quantitative morphometry of remyelination.

Chronically infected mice (5 to 6 months after infection) were given intraperitoneal injections of mAb twice weekly for 5 weeks (50 μg/injection). Total dose of each Ab was 0.5 mg. Mice were sacrificed 2 weeks after the completion of mAb treatment. Light microscopic sections were prepared as described[10]. Mice were anesthetized with ether, exsanguinated by cardiac puncture, and fixed by intracardiac perfusion with Trump's fixative (phosphate-buffered 4% formaldehyde containing 1.5% glutaraldehyde, pH 7.2). The entire spinal cord was removed and sectioned into 1 mm transverse blocks. Every third block was postfixed in 1% osmium tetroxide and embedded in Araldite (Polysciences). One μm sections were cut and stained with *p*-phenylenediamine. Ten spinal cord sections of each mouse were examined. Remyelination and demyelination on each section were quantitated using a Zeiss interactive digital analysis system. Abnormally thin myelin sheaths relative to axon diameter was used as the criterion for remyelination by oligodendrocytes.

Cell culture and immunocytochemistry

Oligodendrocytes, astrocytes and microglia were isolated from telencephalon of newborn Sprague-Dawley rats as described (13). Abs were diluted in PBS. Surface staining was carried out at 4°C for 15 min on unfixed cells after blocking with PBS containing 3% normal goat serum (NGS). Cytoplasmic antigen staining was carried out on cells fixed for 10 min at 4°C with 2% paraformaldehyde and treated for 5 min at room temperature with 0.05% saponin in PBS followed by blocking with 3% NGS in PBS. After incubation with the primary Abs and the secondary FITC-conjugated anti-mouse IgM (μ chain specific) Ab (Jackson Immunoresearch), the slides were mounted in MOWIOL® (Aldrich Chemical) containing 2.5% 1,4-diazobicyclo-[2.2.2]-octane (DABCO, Sigma) and viewed with an epifluorescent microscope.

Direct ELISA

Protein antigens including human RBC spectrin, bovine myosin (heavy chain), mouse albumin, mouse hemoglobin, mouse transferrin, hen egg lysozyme (HEL), rabbit actin, rabbit myelin basic protein (MBP), and keyhole limpet hemocyanin (KLH) were purchased from Sigma. Proteins were tested for purity by SDS-polyacrylamide gel electrophoresis. All chemical haptens were purchased from Sigma and coupled to bovine serum albumin (BSA) (11). Protein antigens were used at 5 μg/ml, and haptens at 2 μM. The proteins and hapten-BSA antigens were coated onto polystyrene or polyvinylchloride microtiter plates in 0.1 M carbonate buffer, pH 9.5, for 18 hours at 4°C. Coated plates were blocked with PBS containing 5% non fat dry milk and 0.05% Tween 20 for 2 hours at room temperature, and incubated with mAbs diluted in blocking buffer (2 μg/ml) for 4 hours at room temperature. TEPC183 (Sigma), and XMMEN-OE5 IgMκ mAbs were used as isotype control Abs. Bound IgM was detected with biotinylated goat anti-mouse IgM (μ chain specific; Jackson Immunoresearch) followed by alkaline phosphatase conjugated to streptavidin, with *p*-nitrophenylphosphate as the chromogenic substrate. Absorbance was determined at 405 nm.

Immunoblotting

Rabbit MBP (Sigma) was separated by SDS-polyacrylamide gel electrophoresis on 15% acrylamide gels. Protein was transferred to a nitrocellulose membrane by electroblotting. The membrane was blocked with Tris buffered saline containing 5% non-fat dry milk and 0.03% Tween 20 for 2 hours at room temperature. The membrane was incubated with SCH79.08, SCH94.03, control IgM (MOPC104E), rabbit polyclonal anti-MBP (1:200, Dako) Abs for 4 hours at room temperature. SCH79.08, SCH94.03, and control IgM were used at the same concentration (10 μg/ml). Bound Abs were detected with biotinylated secondary Abs (Jackson Immunoresearch) and alkaline phosphatase-conjugated streptavidin using 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazorium (BCIP/NBT).

RESULTS AND DISCUSSION

After cell fusion and cloning, a large panel of mAbs were screened by ELISA. One mAb, designated SCH79.08, which belonged to IgMκ subclass showed significant binding to SCH by ELISA. SJL/J mice chronically infected with TMEV and treated with SCH79.08 showed significantly greater CNS remyelination than animals treated with PBS or isotype-matched control mAb (Table 1). On average approximately 20% of the total demyelinated area showed CNS remyelination in mice treated with SCH79.08 ($p < 0.05$), as compared to 2 to 5% in animals treated with PBS or isotype-matched mAb respectively. Treatment with SCH79.08 had no affect or the extent of demyelination (Table 1). Remyelinated lesions were characterized by hundreds of axons with thin myelin sheaths and a relative absence of inflammatory cells or macrophages (Fig. 1). In contrast, most lesions in mice treated with the control IgMκ mAb ABPC22 or PBS had few if any remyelinated axons and the lesions contained intense inflammation (Fig. 1). Even though SCH94.03 and SCH79.08 were selected exclusively for their ability to promote remyelination and not based on antigen specificity, characterization showed that SCH79.08 has remarkable similarities with SCH94.03. Both mAbs a) belong to the IgMκ subclass; b) are multi-organ reactive and polyreactive toward multiple protein antigens (Fig. 3A) and chemical haptens (data not shown); c) stain strongly cytoplasmic structures of most cells by immunofluorescence in a pattern resembling a cytoskeletal protein (Fig. 2B and D); d) recognize oligodendrocyte-specific antigens; SCH79.08 reacting with MBP (Fig. 3A and D) and SCH94.03 binding to an uncharacterized surface antigen on oligodendrocytes (Fig. 2A). In spite of binding similarities, variable region cDNA sequences of SCH79.08 are completely different from those of SCH94.03 (cDNA sequences of SCH79.08 are available from the GenBank database under accession number U91317 and U92070). SCH79.08 VH belonged to VH558 family. The D segment was derived from germline DQ52 gene. The JH region was identical with JH2 germline gene. The Vκ segment for the light chain belonged to Vκ24 family, whereas the Jκ segment was identical with Jκ5 germline gene.

The fact that both SCH94.03 and SCH79.08 bound to oligodendrocytes, but reacted to different antigens on these cells supported the indirect hypothesis of Ab-mediated CNS remyelination. Of interest SCH94.03 and SCH79.08 have important similarities with a number of well-recognized oligodendrocyte-reactive mAbs. Mouse IgMκ mAbs O1, O4 (14), A2B5 (15), and HNK-1 (16) recognize unique differentiation stage-specific surface antigens on oligodendrocytes. O1 recognizes multiple lipid antigens including galactocerebroside, monogalactosyl-diglyceride and psychosine (17), O4 recognizes proligodendroblast antigen and sulfatide (17), and A2B5 recognizes ganglioside GQ1c and other gangliosides (18). The carbohydrate epitope on myelin-associated glycoprotein (MAG) appears to be the principal antigen recognized by HNK-1 (19). This carbohydrate epitope recognized by HNK-1 is also present in other cell adhesion molecules in the nervous system. Similar to SCH94.03 and SCH79.08, these mAbs all belong to IgMκ subclass, are polyreactive, recognize distinct antigens on oligodendrocytes, and recognize intracellular structures of many cell types. In addition variable region cDNA sequences of these mAbs indicated minimal mutations from the germline Ig genes, a characteristic feature of natural autoantibodies (20).

Based on these striking similarities, we tested the therapeutic efficacy of oligodendrocyte-reactive mAbs, O1, O4, A2B5, and HNK-1 in the TMEV model. A mouse IgG3 mAb R24 which recognizes ganglioside GD3 expressed on O-2A progenitor cells and an irrelevant mouse IgMκ mAb (XMMEN-OE5) without reactivity to oligodendrocytes were also tested to determine if Ig isotype and specificity to a unique oligodendrocyte differentiation stage were critical for function. SJL/J mice treated with oligodendrocyte-reactive mAbs O1, O4, A2B5, and HNK-1 showed significantly enhanced CNS remyelination compared to SJL/J mice treated with control IgMκ or PBS (Table 2). Approximately 20 to 24% of the area of demyelination was remyelinated in mice treated with O1, O4, A2B5, and HNK-1. Remyelinated lesions in mice treated with O1, O4, A2B5, and HNK-1 were characterized by axons with thin myelin sheaths relative to axon diameter and a relative absence of inflammation (Fig. 1). In contrast mice treated with R24 did not show significant enhanced remyelination (Table 2, Fig.

1). Immunocytochemical study showed that even though R24 reacts with th surface of O-2A progenitor cells, it does not stain intracellular structures of glial cells (Fig. 2). By ELISA R24 did not react with any of the protein antigens (Fig. 3C) and chemical haptens examined (data not shown) indicating that R24 is not polyreactive in contrast to O1, O4, A2B5, and HNK-1.

Having established that a unique family of oligodendrocyte-specific Abs, each with distinct surface antigen reactivities would induce remyelination, it was critically important to determine whether the complementarity-determining regions (CDR) which form the Ab-binding site is essential. Mouse B cell lymphoma CH12 (CH12.Lx cell) secretes IgMκ mAb under the stimulation with lipopolysaccahride. This secreted IgMκ mAb CH12 is encoded by exactly the same germline Ig genes as the genes of SCH94.03; the nucleotide and amino acid differences between SCH94.03 and CH12 exist only in the CDR3 of heavy and light chains (11). Despite of this structural similarity, CH12 did not stain the surface of oligodendrocytes, did not stain intracellular structure of glial cells (data not shown), and was not polyreactive by ELISA (11). TMEV-infected SJL/J mice treated with CH12 IgMκ mAb did not show significant CNS remyelination compared to control groups (Table 2, Fig. 1). This result establishes that the CDR3 of Ig is critical for the remyelination promoting activity for this family of Abs, and supports the hypothesis that the ability of these Abs to bind within the demyelinated lesions is important to the induction of remyelination.

Despite the fact that all of the mAbs which promoted CNS remyelination were IgMκ, there is no obvious common pattern in germline Ig gene usage of these mAbs. Even though R24 binds to oligodendrocyte progenitors, it did not promote remyelination possibly because of its IgG isotype, lack of polyreactivity by immunocytochemistry and ELISA, or reactivity to cells of an earlier developmental stage. CH12, which has been classified as a natural autoantibody (21), was not widely polyreactive (11), did not bind to oligodendrocytes, and did not promote remyelination. Taken in concert this indicates that only a unique population of polyreactive natural autoantibodies with oligodendrocyte reactivity, irrespective of antigen specificity, are effective for treatment. To this point only Igs of the μ isotypes have induced remyelination,

suggesting the possibility that effector functions preferentially mediated by the μ heavy chain are central to this process.

The mAbs which promote remyelination recognize different differentiation stages of oligodendrocytes from progenitor to mature suggesting that remyelination promoting activity may be independent of developmental stage. We also reported that affinity-purified polyclonal anti-MBP Abs promote CNS remyelination; therefore that reactivity to a marker of mature oligodendrocytes is also effective (22). The direct binding to surviving oligodendrocytes in the lesion could promote their proliferation or dedifferentiation. Alternatively the Abs could block the differentiation of oligodendrocytes to sustain their reactivity with growth factors. There is support from *in vitro* studies for these possibilities. MAb O4 was reported to stimulate differentiation of oligodendrocytes (23). Abs to galactocerebroside cause transmembrane signaling in oligodendrocytes (24). A mAb (R-mAb) which recognizes galactocerebroside, sulfatide, and developmentally regulated unidentified antigen on oligodendrocytes reversibly blocks oligodendrocyte progenitor cell differentiation at late progenitor stage (25). In the presence of R-mAb, mature oligodendrocytes expressing terminally differentiated markers showed retraction of processes, the formation of swollen cells and reduction of the levels of terminally differentiated markers (26). MAG, which is recognized by HNK-1, has been shown to be a major inhibitor of axonal regeneration *in vitro* (27, 28), though its inhibitory activity of axonal regeneration *in vivo* remains inconclusive (29, 30). Possibly HNK-1 may promote CNS remyelination by interfering with MAG expression. However, the observation that multiple oligodendrocyte-reactive Abs each with distinct antigen specificities promote remyelination is most consistent with the hypothesis that direct binding of the mAbs to injured oligodendrocytes in the lesion induces myelin repair through immune effector mechanism initiated by the μ heavy chain. One possibility is that IgM-binding to damaged cells enhances their removal by scavenger macrophages and microglia so that healthy oligodendrocytes or O-2A progenitor cells can initiate their myelination program.

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FIGURE LEGENDS

Figure 1. Light micrograph demonstrating extensive CNS remy lination following treatment with SCH79.08, O1, O4, A2B5 and HNK-1. Sections are from the spinal cord of SJL/J mice chronically infected with TMEV. Note CNS remyelination, characterized by abnormally thin myelin sheath compared to axon diameter, in demyelinated lesions of mice treated with SCH79.08 (A), O1 (B), O4 (C), A2B5 (D), and HNK-1 (E). Note demyelination without significant remyelination in mice treated with R24 (F), CH12 (G), and control IgMκ ABPC22 (H). Araldite-embedded sections were stained with 1% *p*-phenylenediamine. (Magnification, 875X)

Figure 2. Indirect immunofluorescence of cultured glial cells. Note live surface staining of oligodendrocytes with SCH94.03 (A) and R24 (E), but absence of surface staining with SCH79.08 (C). Note intracellular staining of the cytoplasm of astrocytes with SCH94.03 (B) and SCH79.08 (D), but absence of staining with R24 (F). Scale bar = 20 μm. Oligodendrocytes and astrocytes were isolated from telencephalon of newborn Sprague-Dawley rats.

Figure 3. Protein antigen reactivity of SCH79.08 (A), control IgMκ (XMMEN-OE5)(B) and R24 (C) assessed by direct ELISA. Coated plates were blocked with PBS containing 5% non fat dry milk and 0.05% Tween 20, and incubated with mAbs diluted in blocking buffer. Bound Ig was detected with with biotinylated secondary antibodies followed by alkaline phosphatase conjugated to streptavidin, with *p*-nitrophenylphosphate as the chromogenic substrate. Abbreviations used in these figures: MBP, myelin basic protein; KLH, keyhole limpet hemocyanin; HEL, hen egg lysozyme. No reactivity to these protein antigens or chemical haptens was detected with another control IgMκ mAb (TEPC 183)(data not shown). Western blotting for MBP (D) with SCH79.08. Rabbit MBP was separated on 15% SDS polyacrylamide gels. Bound Ig was detected with biotinylated secondary Abs and alkaline phosphatase-conjugated streptavidin using BCIP/NBT. Molecular weight markers are indicated in kDa at the

left margin. lane 1: control mouse IgM (MOPC104E), lane 2: SCH94.03, lane 3: polyclonal rabbit anti-MBP Ab (Dako), lane 4: SCH79.08. Arrows indicate two MBP isoforms (21.5 and 18.5 kDa) recognized by SCH79.08.

Table 1. Enhancement of CNS remyelination by SCH79.08

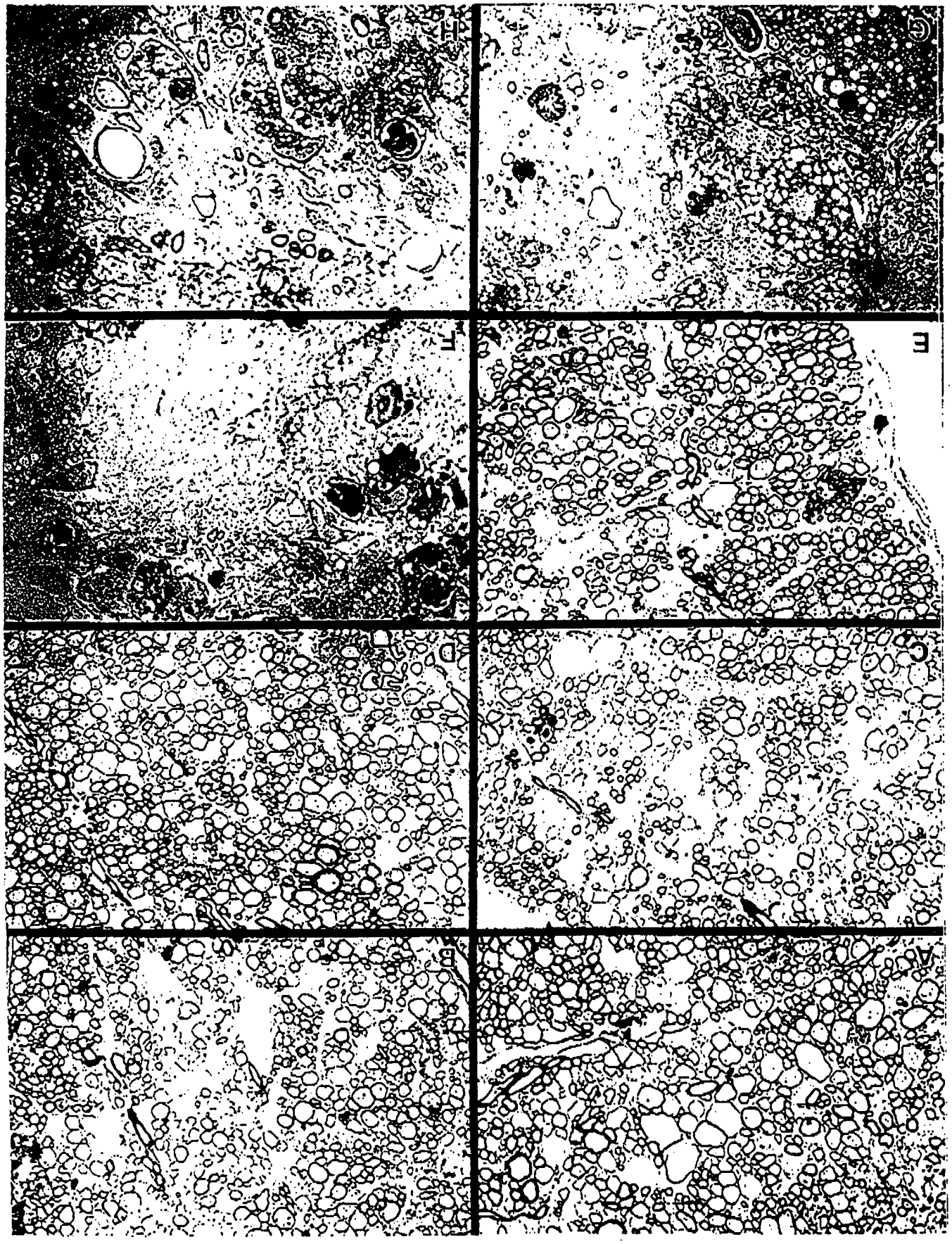
Treatment	No. of mice	Area of white matter (mm^2)	Area of demyelinated lesion (mm^2)	Area of CNS-type remyelination (mm^2)	Area of CNS-type remyelination/area of lesions (%)
SCH79.08	15	8.42 ± 0.33	1.01 ± 0.16	0.20 ± 0.05	20.2 ± 4.7
ABPC22	7	8.06 ± 0.46	1.05 ± 0.22	0.05 ± 0.01	4.9 ± 1.4
PBS	6	8.89 ± 0.26	1.01 ± 0.21	0.03 ± 0.01	2.4 ± 0.8

Values represent the mean ± SEM. Statistics by student t-test of the percent area of CNS-type remyelination/area of lesions in mice treated with SCH79.08 compared to mice treated with ABPC22 (isotype control) or PBS revealed $p < 0.05$.

Table 2. *In vivo* effects of oligodendrocyte-reactive mAbs and CH12 IgMκ mAb

Treatment	No. of mice	Area of white matter (mm^2)	Area of demyelinated lesion (mm^2)	Area of CNS-type remyelination (mm^2)	Area of CNS-type remyelination/area of lesions (%)
O1	6	7.57 ± 0.52	0.53 ± 0.10	0.14 ± 0.04	24.8 ± 6.2*
O4	7	8.01 ± 0.16	0.84 ± 0.10	0.17 ± 0.05	20.4 ± 4.2*
A2B5	7	7.28 ± 0.38	0.70 ± 0.18	0.18 ± 0.05	24.6 ± 4.6*
HNK-1	7	7.16 ± 0.38	0.78 ± 0.10	0.15 ± 0.03	20.6 ± 2.8**
CH12	7	7.43 ± 0.28	0.57 ± 0.11	0.05 ± 0.02	8.5 ± 4.6
R24	7	7.52 ± 0.34	0.65 ± 0.13	0.04 ± 0.01	6.7 ± 2.5
XMMEN-OE5	6	9.32 ± 0.42	1.06 ± 0.27	0.03 ± 0.01	3.4 ± 1.0
PBS	6	7.46 ± 0.76	0.51± 0.15	0.05 ± 0.02	8.0 ± 2.2

Values represent the mean ± SEM. Statistics by student t-test of the percent area of CNS-type remyelination/area of lesions in mice treated with oligodendrocyte-reactive mAbs (O1, O4, A2B5, and HNK-1) compared to mice treated with XMMEN-OE5 (control IgM) or PBS revealed * $p < 0.05$, ** $p < 0.01$. R24 is an IgG3 mAb which recognizes ganglioside GD3 expressed on O-2A progenitor cells. CH12 is encoded by the same germline Ig genes of SCH94.03, and the amino acid differences between them exist only in the CDR3.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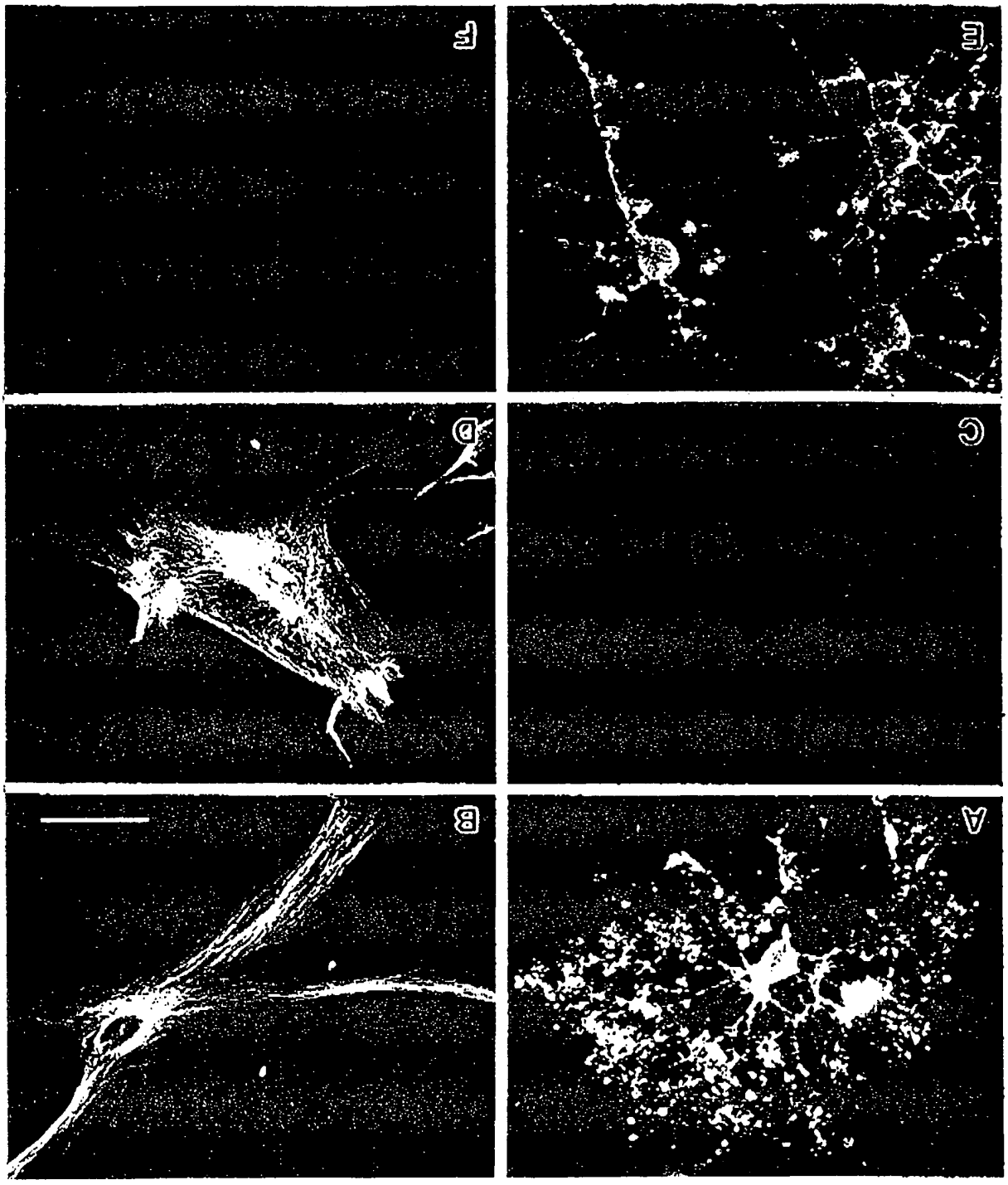
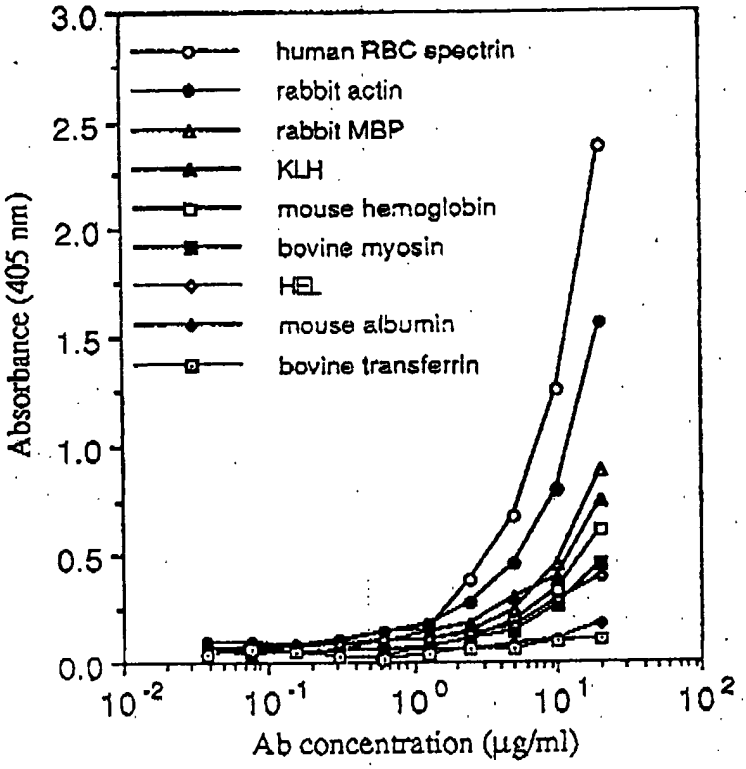
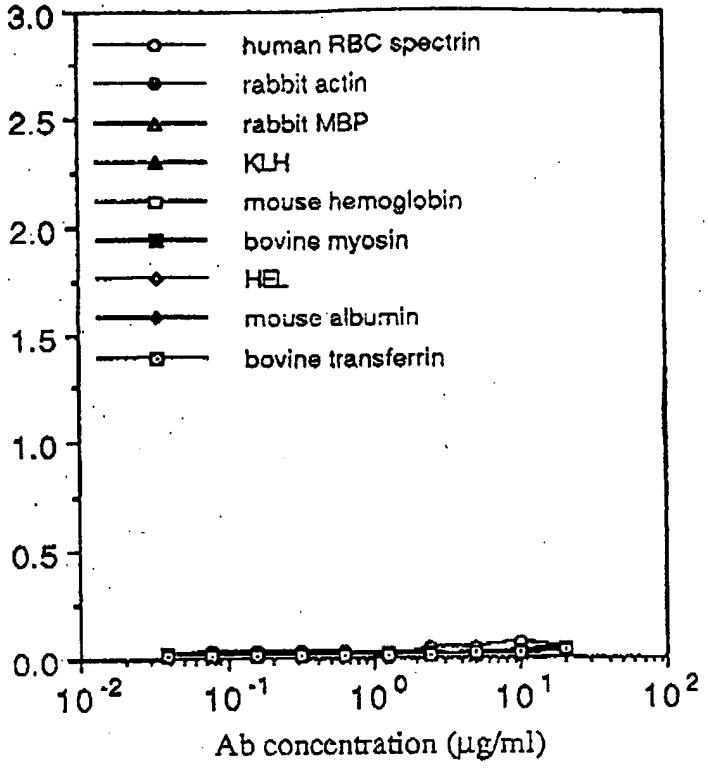

F
E
D
C
B
A

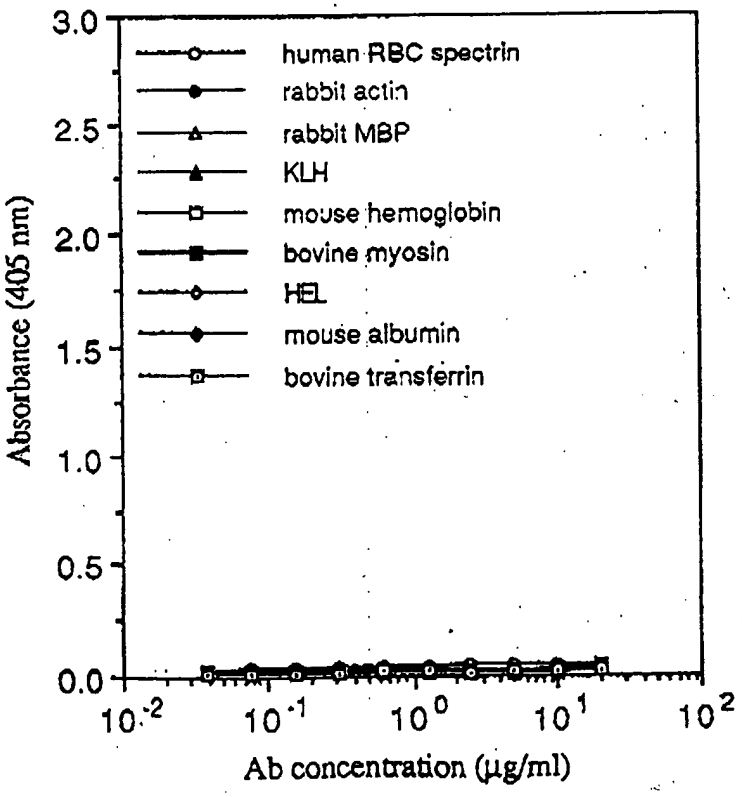
A. SCH79.08



B. control IgM (XMMEN-OE5)



C. R24



D. Western blotting with SCH79.08 for MBP